Journal of Affective Disorders 364 (2024) 65–79

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Research paper

Plasma homocysteine and longitudinal change in cognitive function among urban adults

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ARTICLE INFO

Keywords:
Homocysteine
Cognitive function
Health disparities
Longitudinal study
Older adults

ABSTRACT

Background: Cross-sectional and longitudinal studies have inconsistently linked cognitive performance and change over time to an elevated level of homocysteine (Hcy), with few conducted among urban adults.
Methods: Longitudinal data [Visit 1 (2004–2009) and Visit 2 (2009–2013)] were analyzed from up to 1430 selected Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) participants. Baseline and follow-up blood Hcy was measured, while 11 cognitive function test scores were assessed at either of these two visits. Overall, sex- and race-stratified associations were evaluated using mixed-effects linear regression models, adjusting for key potential confounders. Interaction effects between Hcy and serum levels of folate and vitamin B-12 were also tested.
Results: We found that greater $LnHcy_{v1}$ was significantly associated with poorer baseline attention based on higher Log_e (TRAILS A, in seconds) [β (SE): 0.101 (0.031), $P = 0.001$]. Heterogeneity was also found by sex and by race. Most notably, among men only, $LnHcy_{v1}$ was associated with faster decline on the BVRT (# of errors), a measure of visuo-spatial memory (β (SE): 0.297(0.115), $P = 0.010$, reduced model); while among African American adults only, an elevated and increasing LnHcy over time was associated with faster rate of decline on Log_e (TRAILS B, in seconds) [β (SE): +0.012 (0.005), $p = 0.008$], a measure of executive function. Interactions between Hcy, folate and vitamin B-12 blood exposures were also detected.
Conclusions: In summary, sex- and race-specific adverse association between elevated Hcy and cognitive performance over time were detected among middle-aged urban adults, in domains of attention, visuo-spatial memory and executive functioning.

Abbreviations: AD, Alzheimer's disease; AF, animal fluency; AMR, analytical measuring range; BTA, Brief Test of Attention; BVRT, Benton Visual Retention Test (# of errors); CDT, Clock Drawing Test; CMIA, chemiluminescent microparticle immunoassay; CV, coefficient of variation; CVLT-List A, California Verbal Learning Test Immediate Recall (List A); CVLT-DFR, California Verbal Learning Test, Delayed Free Recall; DS-B, Digit Span Backwards test; DS-F, Digit Span Forward test; GBTM, group-based trajectory models; GM, gray matter; HANDLS, Healthy Aging in Neighborhoods of Diversity Across the Life Span; Hcy, homocysteine; Hcy_{traj}, elevated homocysteine trajectory exposure, z-scored probability.; HEI-2010, Healthy Eating Index, 2010 version; IMR, Inverse Mills Ratio; IRP, Intramural Research Program; Ln or Log_e, natural logarithm; $LnHcy_{v1}$, first-visit Hcy, Log_e transformed; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; MRI, magnetic resonance imaging; MRV, medical research vehicle; MTA, Fazekas and temporal lobe atrophy; MTHFR, methylenetetrahydrofolate reductase; OCM, one-carbon metabolism; NIA, National Institute on Aging; SAM, S-adenosylmethionine; SE, standard error; SEM, standard error of the mean; SES, socio-economic status; sVAD, subcortical vascular dementia; traj and trajplot, Stata commands for GBTM; THFR, tetrahydrofolate; TRAILS A, Trail Making Test Part A; TRAILS B, Trail Making Test Part B; V1, Visit 1; V2, Visit 2; WRAT-3, Wide Range Achievement Test, third edition.

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[1] MAB had full access to the data used in this manuscript and completed all the statistical analyses.

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https://doi.org/10.1016/j.jad.2024.08.010
Received 24 January 2024; Received in revised form 5 July 2024; Accepted 9 August 2024
Available online 10 August 2024
0165-0327/Published by Elsevier B.V.

1. Introduction

Hyperhomocysteinemia, or an increased level of the sulfur amino acid Hcy in the plasma, is acknowledged as an independent risk factor for peripheral vascular, cerebral, and cardiovascular disease (Refsum et al., 1998). As a result, there has been speculation about the possible association of hyperhomocysteinemia with older people's cognitive ability. Several studies have also linked high levels of Hcy to an increased risk of incident Alzheimer's Disease (AD) or dementia from all causes. Studies have indicated that Hcy has a selective relationship with cognitive domains (Garcia et al., 2004; Mooijaart et al., 2005; Teunissen et al., 2003). As Hcy may be more prevalent in some regions of the brain than others, research has connected Hcy to higher levels of white matter hyperintensities as well as brain atrophy (Bleich and Kornhuber, 2003; den Heijer et al., 2003; Dufouil et al., 2003; Sachdev et al., 2002; Scott et al., 2004).

Although blood Hcy levels rise with age and reduced kidney function, key determinants of these levels are dietary intakes of various influential B-vitamins (primarily B-2, B-6, B-9 (also known as folate) and B-12) as well as genetic risk markers including those associated with the *MTHFR* gene (Bottiglieri, 2005). These nutrients are necessary for the methylation events that turn Hcy into methionine and cysteine (Bottiglieri, 2005). Thus, dietary changes that increase B-vitamin consumption can potentially reduce plasma Hcy concentrations. Furthermore, studies examining the relationship between Hcy and cognitive functioning also tested effects of B-vitamins. It has been demonstrated that the plasma level of vitamin B-12 is negatively associated to that of Hcy (Selhub et al., 1993). Studies have demonstrated that vitamin B-12 may be inversely related to cognitive decline (Duthie et al., 2002; Kado et al., 2005; Tucker et al., 2005). At least five other studies concluded similarly that folate is inversely linked to cognitive deterioration or impairment (Duthie et al., 2002; Feng et al., 2006; Kado et al., 2005; Mooijaart et al., 2005; Ramos et al., 2005; Ravaglia et al., 2005; Tucker et al., 2005). Two further trials indicated a protective benefit for vitamin B-6 (Kado et al., 2005; Tucker et al., 2005). Antagonistic interactions of vitamin B-12 and folate with Hcy in its association with cognition have also been observed (Haan et al., 2007; Li et al., 2008; Vidal et al., 2008). Hcy was demonstrated to have neurotoxic and excitotoxic qualities in vitro,(Kruman et al., 2000; Parsons et al., 1998) adding biological plausibility to a direct causal relationship with poor cognition, in addition to its association with cardiovascular disease.

Nineteen selected cohort studies linking Hcy to the various cognitive outcomes were included in a comprehensive review and meta-analysis of various modifiable risk factors (Beydoun et al., 2014). Of these, 12 (63.2 %) found the relationship in the predicted direction for most outcomes of interest (Clarke et al., 2007; Ford et al., 2012a; Ford et al., 2012b; Haan et al., 2007; Kim et al., 2008b; Quadri et al., 2005; Ravaglia et al., 2005; Seshadri et al., 2002; Tucker et al., 2005; van den Kommer et al., 2010; van Raamt et al., 2006; Zylberstein et al., 2011). Comparably, out of the 14 cross-sectional studies that were chosen, 11 (or 78 %) discovered an association between most outcomes of interest in the predicted direction,(Duthie et al., 2002; Elias et al., 2006; Feng et al., 2006; Kim et al., 2007; Kim et al., 2008a; Miller et al., 2003; Perneczky et al., 2011; Prins et al., 2002; Ramos et al., 2005; Ravaglia et al., 2003; Schafer et al., 2005). Focusing on AD incidence, our meta-analysis indicated that the percentage population attributable risk for elevated vs. low Hcy on AD incidence was estimated at 21.7 % [95%CI: 12.8–30.6] (Beydoun et al., 2014). Since 2014, more up to date studies added to the evidence of an adverse effect of elevated Hcy along with its associated B-vitamin deficiencies on various cognitive and brain magnetic resonance imaging (MRI) outcomes (e.g.(Ansari, 2016; Behrens et al., 2020; Beydoun et al., 2020d; Chang et al., 2023; Gong et al., 2022; Lanyau-Dominguez et al., 2020; Luzzi et al., 2022; Ma et al., 2017; Moretti et al., 2017; Nelson et al., 2021; Przybycien-Gaweda et al., 2022; Raszewski et al., 2016; Seema et al., 2023; Silberstein et al., 2022; Smith et al., 2018; Song et al., 2022; Wang et al., 2022; Zhang et al., 2022; Zhang et al., 2023)), though none thus far have examined this relationship specifically among White and African American middle-aged adults residing in urban areas within the United States, using multiple repeats on both homocysteine and cognitive measures. Such urban areas are known to have diverse populations in terms of racial and ethnic composition as well as socio-economic status, with a large proportion living below poverty. It is important to examine these association across sex and race, in particularly, given the known genetic basis for hyperhomocysteinemia that is strongly determined by the *MTHFR* gene variants, with some related single nucleotide polymorphisms being rare among individuals of African ancestry(Beydoun et al., 2019c). Furthermore, sex differences are well-known for blood homocysteine, with concentrations being on average consistently higher among men according to US national data (Beydoun et al., 2020a; Beydoun et al., 2010).

In this longitudinal study, we examined baseline and trajectories in blood Hcy between visit 1 (v1: 2004–2009) and visit 2 (v2: 2009–2013), as well as their relationships with cognitive function test scores (at baseline and change between visits 1 and 2), while examining health disparities according to sex and race. This was accomplished by performing secondary analyses of v_1 and v_2 data from the Healthy Aging in Neighborhoods of Diversity across the Lifespan Study (HANDLS). We hypothesized a time-dependent relationship between rising Hcy and declining cognitive function, with differentials across racial and sex groups, given the previous evidence of a cross-sectional relationship as well as an association between baseline Hcy and age-related cognitive decline.

2. Materials and methods

2.1. Database

The National Institute on Aging (NIA) Intramural Research Program (IRP) launched the HANDLS study in 2004. It is an ongoing prospective cohort study with the goal of addressing health disparities in age-related disorders. A unique multidisciplinary research study, HANDLS looks at a wide range of factors in higher and lower socioeconomic status (SES) groups of African American and White individuals. To increase participation rates and retention among non-traditional research participants, it makes use of cutting-edge research instruments and mobile medical research vehicles (MRVs). The National Institutes of Health's Institutional Review Board authorized the HANDLS project, and study participants gave written informed consent (Beydoun et al., 2020b; Beydoun et al., 2019b; Beydoun et al., 2019d; Evans et al., 2010; Hossain et al., 2019; Kuczmarski et al., 2015; Wendell et al., 2016; Wright et al., 2019; Wright et al., 2017; Beydoun et al., 2023a) .

Two phases of baseline HANDLS data collection (v1) took place between 2004 and 2009. In the first phase, the participants were interviewed in-home and given questionnaires regarding their health, use of health services, psychosocial factors, nutrition, neighborhood features, and demography. The second phase, which was carried out in MRVs, comprised laboratory measurements (blood chemistries, hematology, biomarkers of oxidative stress, biomaterials for genetic studies), medical history, physical examination, dietary recall, cognitive evaluation, and psychophysiological assessments (heart rate variability, arterial thickness, carotid ultrasonography, assessments of muscle strength, bone density). Participants in HANDLS were followed every five years, with v2 occurring between 2009 and 2013. There are both one-time and specialized assessments conducted during HANDLS visits. HANDLS metadata are available at https://handls.nih.gov/06Coll-w00dataDocR.cgi.

2.2. Measures

2.2.1. Homocysteine

Aeon Technologies, LLC tested Hcy using the Alinity I analyzer for

serum quality, detecting icteria, lipemia, and hemolysis. The Alinity i Homocysteine assay was used to quantify Hcy, with an analytical measuring range of 1.00 to 50.00 mmol/L. Twelve batches of samples were processed, with a serum sample as a control. The main exposure variable was Hcy measured at v1 of the HANDLS study, Log_e transformed (LnHcyv1). A STATA plugin was used to identify groups of individuals with comparable developmental trajectories throughout time (Jones, 2001; Jones, 2007). This group-based method uses maximum likelihood and a multinomial modeling strategy to estimate model parameters. The quasi-Newton procedure was used to optimize the results. Group-based trajectories over time were presented with 95 % confidence intervals and specified a censored normal distribution for the chosen outcomes.

2.2.2. Cognitive function

At visits 1 and 2 of the HANDLS investigation, 11 cognitive test scores were used to identify the major outcome variables. The Mini-Mental State Examination (MMSE), the California Verbal Learning Test (CVLT) Immediate (List A) and Delayed Free Recall (DFR), the Benton Visual Retention Test (BVRT, # of errors), the Brief Test of Attention (BTA), the Animal Fluency test (AF), the Digit Span Forward and Backwards tests (DS-F and DS-B), the Clock Drawing Test (CDT), and the Trail Making Test Part A and B (TRAILS A and B, in seconds) were among the tests used by clinical staff to assess cognition (Beydoun et al., 2023b). Supplemental Material 1 has a thorough explanation of every cognitive exam. Global mental state, verbal memory, verbal fluency, attention, visual memory, visuospatial ability, and executive function, which includes working memory, were among the cognitive domains covered. Using previously mentioned techniques (Philipps et al., 2014), the total MMSE score was normalized. To obtain pseudo-normality, the Trails A and B scores (in seconds) were Log_e-transformed. All cognitive test scores, with the exception of BVRT, Trails A and B, were coded in the direction of higher values, indicating improved performance during Visits 1 and 2.

2.2.3. Covariates

Potential confounders included demographics [sex (male, female), age (years), race (White, African American), and poverty status (<125 % federal poverty line = 1, ≥ 125 % federal poverty line, ≥ 125 % federal poverty line = 0), education (less than high school, high school, more than high school), literacy (Wide Range Achievement Test, third edition (WRAT-3) (Supplemental material 1)); lifestyle factors [current cigarette smoking (Yes = 1, 0 = No)], current drug use (Yes = 1 for using any of marijuana, opiates, and cocaine, No = 1), and the 2010 Healthy Eating Index [HEI-2010] and health-related factors (body mass index [BMI; weight/height2 in kg.m-2, continuous], comorbidities, depression symptoms score and self-rated health). The age at visit 1 and 2 was used to calculate the period between visits, while the age at visit 1 was included as a continuous covariate. Poverty status was operationalized using Department of Health and Human Services poverty levels based on household income and total household size (Department of Health and Human Services, 2004). The HEI-2010 assessed the general quality of a subject's diet (Beydoun et al., 2020c, 2023). Comorbidities were operationalized as four binary/categorical covariates: self-reported history of cardiovascular diseases, dyslipidemia, diabetes, and hypertension. The Center for Epidemiological Studies Depression Scale was used to assess depressive symptoms (Supplementary material 2) The final classification for self-rated health was 0 for poor/average, 1 for good, and 2 for very good/excellent. Nutritional biomarkers, mainly serum folate and vitamin B-12, were considered as potential effect modifiers in parts of the analysis. Participants were instructed to fast for at least 8 h before MRV visits, and serum cobalamin (i.e. vitamin B-12) and folate were tested using Quest Diagnostics (Ispir et al., 2015; Owen and Roberts, 2003). Dietary total folate and vitamin B-12 were included among covariates in other secondary analyses, also based on the average of two 24 h dietary recalls at v_1. Given that co-morbidities, depressive symptoms and other health-related factors such as self-rated health may be mediating the association between Hcy and cognitive performance and change over time, only a sensitivity analysis was used to adjust for these covariates in addition to BMI, lifestyle and socio-demographic factors. The directed acyclic graphs with and without these potential mediators is shown in Supplementary material 3, suggesting that Model 3 may be incorrectly adjusted.

2.3. Statistical methods

STATA version 18 was used for all statistical analyses (StataCorp, College Station, TX). Measures of dispersion (standard deviation, interquartile range) and central tendency (mean, median) for continuous variables were included in summary statistics, together with counts and percentages for categorical variables. Bivariate linear regression models were used to investigate associations between $LnHcy_{v1}$ tertiles (ordinal predictor) and continuous study characteristics of interest as outcomes. Bivariate linear and multinomial logistic regression models were also used to test the association between $LnHcy_{v1}$ tertiles and various categorical characteristics, while considering T_1 as the common referent for the main tertile predictor variable.

The *mixed* command in Stata was used to fit mixed-effects linear regression models, which are valuable for examining longitudinal data (StataCorp, 2023). Using complete case analysis, *mixed* handles missing data on the outcome variable, by including only subjects with at least one observation on the outcome variable (StataCorp, 2023). The *mixed* command is particularly effective for managing unbalanced data, a common occurrence in longitudinal research (StataCorp, 2023). However, this process can result in data loss and biases if the absence of data is not entirely random (StataCorp, 2023). The construction of mixed-effects regression models involved a sequential examination of socio-demographic, lifestyle and health-related factors as potential confounders (Supplemental Material 4). Testing for multicollinearity among the variables included in mixed-effects models was one of the model-building procedures. Given that each covariate had <5 % missing data on average, we ensured sample sizes were constant between different adjusted models by performing multiple imputations (5 imputations, 10 iterations) using the chained equations methodology in order to reduce missing data caused by the addition of covariates into different models. During the imputation, all covariates were employed simultaneously similarly with other research (Beydoun et al., 2016a, 2019a, 2023b). More specifically, *mi unregister/register*, *mi impute*, *mi passive* and *mi estimate* were the main sub-commands used to obtain the multiple imputed data and estimate various parameters across these imputations using Rubin's rule.

First, using the largest sample after excluding HANDLS subjects with missing MMSE data, baseline sociodemographic, lifestyle, and health characteristics, baseline LnHcy and Hcy_{traj}, as well as cognitive test scores (at baseline and change over time) were described before and after stratification according to baseline LnHcy tertiles. Subsequently, different sets of covariates were considered when building a series of mixed-effects linear regression models for baseline Hcy (Log_e transformed or LnHcy) as a predictor of cognitive test scores (at baseline and change over time) and Hcy trajectories as a predictor of cognitive test scores (at baseline and change over time). The research period, measured in years, between visits 1 and 2, was the time variable that was employed. Age, sex, race, poverty status, inverse mills ratio (IMR), time on study, and its interaction with variables and Hcy exposures were all adjusted for in Model 1. Models 2 were adjusted for duration on study between visits 1 and 2, its interaction with $LnHcy_{v1}$ or Hcy_{traj} and variables, age, sex, race, poverty status, education, literacy, smoking, drug use, the 2010 HEI, BMI, and IMR. Model 3 further adjusted Model 2 for hypertension, diabetes, dyslipidemia, cardiovascular disease, depressive symptoms and self-rated health (sensitivity analysis). For Models 1 and 2, the interaction effects of $LnHcy_{v1}$ or Hcy_{traj} with sex and race were assessed, and stratified analyses were carried out independently for

White, African American participants, men and women. Therefore, we used Models 1–2 on two exposures ($LnHcy_{v1}$ or Hcy_{traj}), two stratifying variables (sex, race), and 11 cognitive test scores with a maximum of two repeats (impact on baseline and change in cognitive test scores). Using a two-stage Heckman selection technique, we corrected for sample selectivity resulting from missing data in all models, particularly accounting for differences between those selected for this study and those excluded out of the total target HANDLS population. We estimated all mixed-effects linear regression models while adjusting for a predictor for sample selection known as the inverse mills ratio (IMR), a function of the conditional probability of being selected on age, sex, race, and poverty status, in addition to the previously mentioned covariates (Beydoun et al., 2013, 2023b). Prior to multiple testing correction, the type I error rate for the main and interaction effects was predetermined to be 0.05 and 0.10, respectively (Selvin, 2004). Beyond this point, we used the familywise Bonferroni correction (Hochberg and Tamhane, 1987) technique to adjust for outcome multiplicity only (i.e., 11 cognitive test scores) and focusing mainly on the reduced Model 1. Then, with the inclusion of possibly confounding and/or mediating variables, Models 2 and 3 were considered as sensitivity models. We therefore adjusted the significant thresholds for the main effects to $p < 0.00455$ (0.05/11) and the two-way interaction terms to $0.10/11 = 0.00910$. This approach was previously applied in other comparable studies with the same types and numbers of cognitive measures (Beydoun et al., 2016b, 2019d, 2023a,b). Effect modification by serum folate and vitamin B-12 (Log_e transformed, z-scored) was assessed as a secondary analysis, by adding 2-way and 3-way interaction terms with each of these covariates, for the overall sample, using an incremental adjustment for other covariates (Models 1–3). Predictive margins of selected cognitive performance outcomes were visualized across time and key Hcy exposures to illustrate the main findings (mainly Model 1, overall). Furthermore, dietary total folate and vitamin B-12 were included in our analysis for descriptive purposes. The full Stata script will be provided on github at: https://github.com/baydounm/HANDLS_HCY_COGN.

3. Results

3.1. Study sample characteristics by Hcy levels

The HANDLS study recruited an initial sample of 3720 participants, with 54.7 % female and mean age 48.3 years. 2468 participants completed the follow-up visit (v_2). Hcy_{v1} data was complete among 1460 participants and 1428 for Hcy_{traj} (Fig. 1) After restricting the study to HANDLS participants with complete and credible cognitive test scores, the final study samples ranged from 1365 to 1446, depending on test scores and Hcy exposure completeness. It is worth noting that for most cognitive test scores, <2 % of the sample was dropped per visit due to non-credible test scores, mainly due to low literacy or a physical disability.

Table 1 presents statistics on baseline socio-demographic, lifestyle, health characteristics, Hcy exposures, cognitive test scores, and annualized changes in cognitive test scores among 1430 study-eligible HANDLS participants with complete MMSE test scores at baseline. The mean (±SEM) $LnHcy_{v1}$ and Hcy_{traj} (probability of belonging to higher Hcy group) were 2.150 (±0.009) and + 0.102 (±0.006), respectively. Differences were detected across $LnHcy_{v1}$ tertiles, including by WRAT total score which was lower at higher $LnHcy_{v1}$ levels. More notably individuals with higher $LnHcy_{v1}$ were older and more likely to be men. They also had lower HEI-2010 total score, higher proportions with hypertension, and higher mean baseline BVRT test score, independently of age, sex, race and poverty status. Additionally, $LnHcy_{v1}$ was inversely related to serum folate, dietary folate and serum B-12 levels, independently of these same socio-demographic and economic factors.

3.2. Mixed-effects linear regression models findings for Hcy-Cognition associations in overall sample

Table 2 displays findings from mixed-effects linear regression models focusing on the associations of $LnHcy_{v1}$ with 11 cognitive test scores (baseline and between-visit annualized change). After adjustment for multiple testing and in the overall sample, baseline LnHcy was significantly but only cross-sectionally associated with Log_e (TRAILS A) (β

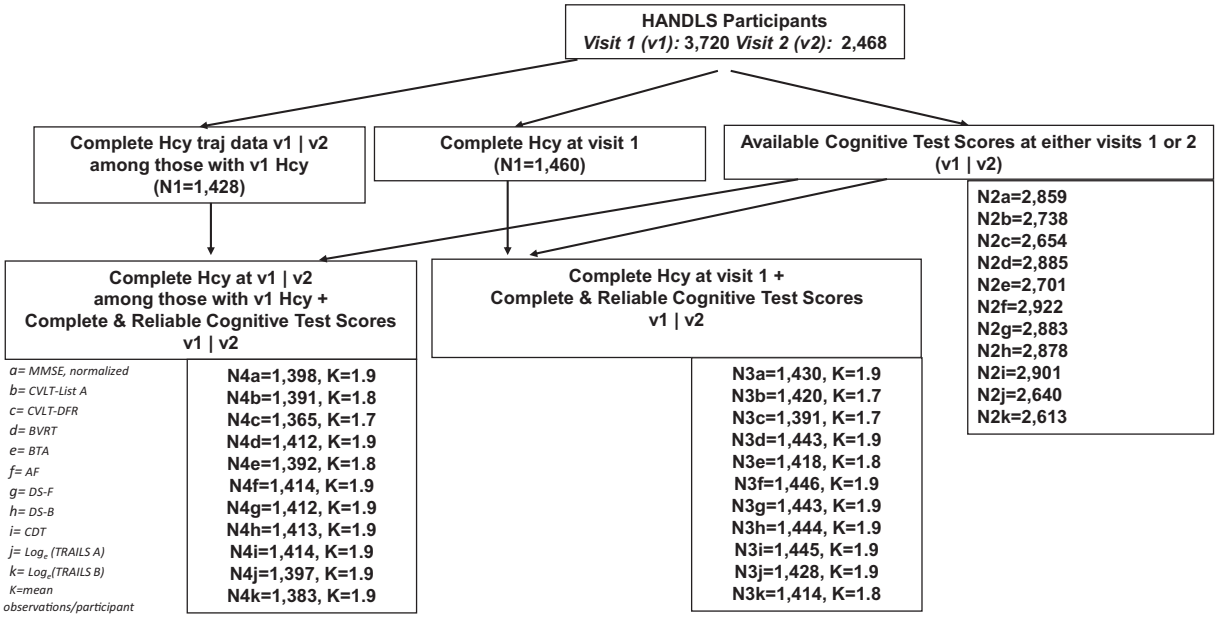


Fig. 1. Study Flowchart – HANDLS (2004–2013).
Notes: HANDLS = Healthy Aging in Neighborhoods of Diversity across the Lifespan Study; Cognitive tests include the Mini-Mental State Examination (MMSE), the California Verbal Learning Test (CVLT) Immediate (List A) and Delayed Free Recall (DFR), the Benton Visual Retention Test (BVRT, # of errors), the Brief Test of Attention (BTA), the Animal Fluency test (AF), the Digit Span Forward and Backwards tests (DS-F and DS-B), the Clock Drawing Test (CDT), the Trail making test Part A and B (TRAILS A and B, in seconds). N = sample size; K = mean observations/participant.

Table 1
Summary statistics for baseline socio-demographic, lifestyle and health characteristics, Log_e transformed blood homocysteine (LnHcy) and cognitive test scores as well as between-visit change in cognitive test scores and Hcy trajectory (Hcy_{traj}) overall, and according to tertiles of baseline LnHcy at v_1 (n = 1430): HANDLS 2004–2013[1].

	N (%) or Mean ± SEM	$LnHcy_{v1}$ tertiles		
		1st	2nd	3rd
	N = 1430	*N* = 476	*N* = 479	*N* = 475
LnHcy exposures:				
			P_{trend} < 0.001	
$LnHcy_{v1}$	2.150 ± 0.009	1.831 ± 0.006	2.129 ± 0.003[d,***]	2.490 ± 0.013[d,***]
			P_{trend} < 0.001	
Hcy_{traj}[b,c]	+0.102 ± 0.006	+0.0128 ± 0.001	+0.0311 ± 0.0044	+0.2646 ± 0.0160[d,***]
Socio-demographic:				
Sex:				
Male	42.4	25.6	42.8[d,***]	40.2[d,***]
Female	57.6	75.4	57.2	59.8
Age (years):			P_{trend} < 0.001	
Continuous	47.9 ± 0.2	45.7 ± 0.4	48.3 ± 0.4[d,***]	49.8 ± 0.4[d,***]
Race:				
White	43.4	44.5	43.4	42.1
African American	56.6	55.5	56.6	57.9
Poverty status:				
<125 % federal poverty line	36.7	37.2	36.1	36.8
≥ 125 % federal poverty line	63.3	62.8	63.9	63.2
Education:				
Less than high school	6.1	6.1	5.9	6.4
High school	56.9	55.6	56.3	58.7
More than high school	37.0	38.2	37.8	35.0
Literacy:			P_{trend} = 0.006	
WRAT-3 score	42.8 ± 0.2	43.5 ± 0.4	42.9 ± 0.3	42.1 ± 0.4[d,**]
Lifestyle:				
Cigarette smoking:				
Yes	43.6	40.3	43.6	47.0
No	56.4	59.7	56.4	53.0
Drug use:				
Yes	18.3	15.1	19.5	20.3*
No	81.7	84.9	80.5	79.7
HEI-2010 score:			P_{trend} = 0.002	
	43.1 ± 0.3	44.5 ± 0.6	43.2 ± 0.6	41.5 ± 0.6[d,**]
Health:				
Body mass index (kg/m^2):			P_{trend} = 0.38	
	29.9 ± 0.2	30.1 ± 0.3	30.0 ± 0.3	29.7 ± 0.3
Self-rated health:				
Poor/Average	21.2	19.3	19.0	25.4
Good	38.9	39.1	39.0	38.6
Very good/ Excellent	39.9	41.5	42.0	36.0
CES-D:			P_{trend} = 0.81	
	14.0 ± 0.3	14.3 ± 0.5	13.2 ± 0.5	14.5 ± 0.5[d]
Hypertension:				
Yes	40.1	32.6	41.7**	46.1[d,***]
No	59.9	67.3	58.3	53.9
Diabetes:				
None	68.0	72.1	68.9	63.1
Pre-diabetes	17.9	15.0	18.0	20.7*
Diabetes	14.1	12.9	13.2	16.2
Dyslipidemia:				
Yes	24.1	20.8	23.8	27.7*
No	75.9	79.2	76.2	72.2
Cardiovascular disease:				
Yes	14.5	15.8	13.7	14.0
No	85.5	84.2	86.2	86.0
Cognitive tests: [a]				
Visit 1				
MMSE total score:				
			N = 1430; P_{trend} = 0.011	
Normalized	77.3 ± 0.4	78.3 ± 0.7	78.0 ± 0.7	75.7 ± 0.7*
			N = 1430; K = 0.008	
Raw	27.8 ± 0.06	28.0 ± 0.10	27.9 ± 0.1	27.6 ± 0.1**
			N = 1185; P_{trend} = 0.026	
CVLT-List A	24.7 ± 0.2	25.5 ± 0.3	24.3 ± 0.3*	24.4 ± 0.3*
			N = 1153; P_{trend} = 0.050	
CVLT-DFR	7.4 ± 0.1	7.7 ± 0.2	7.3 ± 0.2	7.3 ± 0.2*
			N = 1435; P_{trend} = 0.022	
BVRT	6.3 ± 0.1	5.9 ± 0.2	6.3 ± 0.2	6.6 ± 0.2[d,*]
			N = 1205; P_{trend} = 0.008	
BTA	6.80 ± 0.06	7.01 ± 0.11	6.80 ± 0.10	6.61 ± 0.11**
			N = 1427; P_{trend} = 0.27	
AF	19.0 ± 0.1	19.3 ± 0.3	18.9 ± 0.2	18.9 ± 0.2
			N = 1422; P_{trend} = 0.13	
DS-F	7.30 ± 0.05	7.40 ± 0.10	7.40 ± 0.10	7.18 ± 0.10
			N = 1412; P_{trend} = 0.047	
DS-B	5.70 ± 0.06	5.81 ± 0.10	5.7 ± 0.1	5.5 ± 0.1*
			N = 1432; P_{trend} = 0.34	
CDT	8.80 ± 0.03	8.86 ± 0.06	8.83 ± 0.05	8.78 ± 0.05
			N = 1418; P_{trend} < 0.001	
Log_e (TRAILS A)	3.40 ± 0.01	3.40 ± 0.02	3.47 ± 0.02**	3.51 ± 0.02***
			N = 1406; P_{trend} = 0.001	
Log_e (TRAILS B)	4.60 ± 0.02	4.50 ± 0.03	4.59 ± 0.03*	4.65 ± 0.03**

(*continued on next page*)

Table 1 (*continued*)

	N (%) or Mean ± SEM	$LnHcy_{v1}$ tertiles		
		1st	2nd	3rd
	N = 1430	*N* = 476	*N* = 479	*N* = 475
Visit 1 to Visit 2: Empirical bayes estimator of slopes				
MMSE total score:			N = 1430	
Normalized	−0.186 ± 0.000	_	_	_
			N = 1430; P_{trend} = 0.029	
Raw	−0.0127 ± 0.004	−0.0179 ± 0.005	−0.0198 ± 0.005	−0.0003 ± 0.0063*
			N = 1420; P_{trend} < 0.001	
CVLT-List A	−1.136 ± 0.001	−1.130 ± 0.002	−1.139 ± 0.002***	−1.140 ± 0.002***
			N = 1391; P_{trend} < 0.001	
CVLT-DFR	−0.3905 ± 0.0003	−0.3887 ± 0.0006	−0.3911 ± 0.0005**	−0.3917 ± 0.0006***
			N = 1443; P_{trend} = 0.20	
BVRT	+0.4264 ± 0.0124	+0.4059 ± 0.0209	0.4288 ± 0.0220	+0.4447 ± 0.0217
			N = 1418; P_{trend} = 0.018	
BTA	−0.0580 ± 0.0006	−0.0570 ± 0.0009	−0.0569 ± 0.0010	−0.0601 ± 0.0009*
			N = 1446; P_{trend} = 0.22	
AF	+0.0312 ± 0.0000	+0.0312 ± 0.0001	+0.0313 ± 0.0001	+0.03111 ± 0.0001
			N = 1443; P_{trend} = 0.072	
DS-F	−0.0138 ± 0.0003	−0.0134 ± 0.0006	−0.0133 ± 0.0006	−0.0148 ± 0.0006
			N = 1444; P_{trend} = 0.007	
DS-B	−0.0209 ± 0.0002	−0.0217 ± 0.0005	−0.0212 ± 0.0005	−0.0198 ± 0.0005**
			N = 1445; P_{trend} = 0.13	
CDT	−0.0170 ± 0.0007	−0.0160 ± 0.0011	−0.0165 ± 0.0013	−0.0187 ± 0.0013
			N = 1428; P_{trend} < 0.001	
Log_e (TRAILS A)	+0.0055 ± 0.0001	+0.0046 ± 0.0002	+0.0054 ± 0.0002*	+0.0064 ± 0.0003***
			N = 1414; P_{trend} = 0.51	
Log_e (TRAILS B)	+0.0047 ± 0.0008	+0.0056 ± 0.0014	+0.0043 ± 0.0015	+0.0042 ± 0.0014
Folate and vitamin B-12				
			N = 1430; P_{trend} < 0.001	
Serum folate	14.65 ± 0.18	16.54 ± 0.30	15.21 ± 0.32[d,**]	12.20 ± 0.28[d,***]
			N = 1430; P_{trend} = 0.001	
Dietary total folate	367.4 ± 6.9	390.2 ± 13.1	376.6 ± 12.0[d]	335.4 ± 10.3[d,***]
			N = 1430; P_{trend} < 0.001	
Serum vitamin B-12	512.1 ± 6.2	571.1 ± 12.5	519.1 ± 10.3[d,**]	445.9 ± 8.3[d,***]
			N = 1430; P_{trend} = 0.17	
Dietary vitamin B-12	5.71 ± 0.26	6.21 ± 0.64	5.78 ± 0.38	5.15 ± 0.38

Abbreviations: Hcy = Homocysteine; HEI = Healthy Eating Index; Ln or Log_e = Log_e transformed; N=Sample size; WRAT = Wide Range Achievement Test; SEM = Standard error of the mean.

[1]Cognitive tests include the Mini-Mental State Examination (MMSE), the California Verbal Learning Test (CVLT) Immediate (List A) and Delayed Free Recall (DFR), the Benton Visual Retention Test (BVRT, # of errors), the Brief Test of Attention (BTA), the Animal Fluency test (AF), the Digit Span Forward and Backwards tests (DS-F and DS-B), the Clock Drawing Test (CDT), the Trail making test Part A and B (TRAILS A and B, in seconds).

[a] Final sample for socio-demographic, lifestyle and health-related factors and folate and vitamin B-12 variables was sample 3a in Fig. 1. For all other analyses, samples 3a-3 k were used (i.e. cognitive performance measures) as well as sample 4a for Hcy_{traj}.

[b] Hcy_{traj} is the "high increasing" trajectory group probability, using a group-based trajectory modeling (GBTM) approach for LnHcy by age.

[c] P-trend for null hypothesis that β = 0 based on bivariate linear models with main predictor being $LnHcy_{v1}$ tertiles entered as an ordinal variable and outcomes being continuous characteristics. *P*-value for categorical characteristics was derived from a chi-square test with $LnHcy_{v1}$ tertiles.

[d] $P < 0.05$ for null hypothesis that β = 0 based on multivariable-adjusted linear or multinomial logit models with referent category for the predictor $LnHcy_{v1}$ tertiles being the lowest tertile (T1). Covariates included were age, sex race and poverty status.

* P < 0.05.

** $P < 0.010$.

*** P < 0.001 for null hypothesis that β = 0 based on bivariate linear or multinomial logit models with referent category for the predictor $LnHcy_{v1}$ tertiles being the lowest tertile (T1).

(SEM): 0.101 (0.031), $P = 0.001$) in *Model 1*, a finding that retained its statistical significance at type I error of 0.05 in *Model 2* (*Fig. 3*). This association was in the expected direction of worse cognitive function with greater baseline LnHcy. Supplementary Table 1 also included a fully adjusted *Model 3*, which in addition to BMI and lifestyle factors, included other health-related factors such as baseline self-rated health, cardiovascular disease, diabetes, hypertension and dyslipidemia. The addition of these measures only slightly attenuated the cross-sectional association between LnHcy and Trails A.

Group-based trajectory models of LnHcy against longitudinal age yielded a "Low increasing" group 1 (89.3 %) and a "High increasing" group 2 (10.7 %) (Fig. 2). The individual-level predicted probabilities from the GBTM were then z-scored and group 2 membership standardized score of the probability (Hcy_{traj}) and entered as the main predictor in the mixed-effects linear regression models as above (Table 3 and Supplementary Table 1). 1 SD increase in Hcy_{traj} was associated with a cross-sectionally higher Log_eTRAILSA (β (SEM): 0.032 (0.010), P = 0.001) in *Model 1*, reflecting poorer performance within the "high increasing" Hcy group, retaining statistical significance in both *Models 2* (Table 3) and the fully adjusted *Model 3* (Supplementary Table 1). It is worth noting that *Model 3* incrementally adjusted *Model 2* for self-reported cardiovascular disease, diabetes, dylsipidemia, hypertension as well as self-rated health and depressive symptoms as measured by the total CES-D score.

Table 2
Relationship of Log_e transformed blood homocysteine at baseline ($LnHcy_{v1}$) with 11 cognitive test scores (baseline and between-visit change), overall, and by stratifying variables: HANDLS 2004–2013.

OVERALL:[c]	Plasma Homocysteine			
	Model 1[a]		Model 2[b]	
	β (SE)	*P* value	β (SE)	P value
MMSE, normalized:	N = 1430, K = 1.9		N = 1430, K = 1.9	
$LnHcy_{v1}$	−1.62 (1.30)	0.21	+0.46 (1.08)	0.67
$LnHcy_{v1}$ × Time	+0.065 (0.284)	0.82	−0.014 (0.281)	0.96
CVLT-List A:	N = 1420, K = 1.7		*N* = 1420, K = 1.7	
$LnHcy_{v1}$	+0.579 (0.570)	0.31	1.159 (0.533)	0.030
$LnHcy_{v1}$ × Time	−0.102 (0.115)	0.38	−0.097 (0.115)	0.40
CVLT-DFR:	N = 1391, K = 1.7		*N* = 1391, K = 1.7	
$LnHcy_{v1}$	+0.292 (0.268)	0.28	+0.535 (0.257)	0.037
$LnHcy_{v1}$ × Time	−0.009 (0.056)	0.88	−0.006 (0.056)	0.92
BVRT:	N = 1443, K = 1.9		*N* = 1443, K = 1.9	
$LnHcy_{v1}$	0.433 (0.404)	0.28	0.031 (0.386)	0.94
$LnHcy_{v1}$ × Time	0.140 (0.081)	*0.085*[e]	+0.120 (0.081)	0.14[e]
BTA:	*N* = 1418, K = 1.8		N = 1418, K = 1.8	
$LnHcy_{v1}$	−0.255 (0.195)	0.19	−0.102 (0.188)	0.59
$LnHcy_{v1}$ × Time	−0.0096 (0.0426)	0.82	−0.000 (0.043)	0.99
AF:	N = 1446, K = 1.9		*N* = 1446, K = 1.9	
$LnHcy_{v1}$	−0.507 (0.449)	0.26[f]	−0.008 (0.428)	0.99[f]
$LnHcy_{v1}$ × Time	−0.032 (0.082)	0.70	−0.038 (0.083)	0.65
DS-F:	N = 1443, K = 1.9		N = 1443, K = 1.9	
$LnHcy_{v1}$	−0.284 (0.182)	0.12	−0.050 (0.166)	0.76
$LnHcy_{v1}$ × Time	−0.027 (0.033)	0.41	−0.024 (0.033)	0.47
DS-B:	N = 1444, K = 1.9		N = 1444, K = 1.9	
$LnHcy_{v1}$	−0.322 (0.180)	0.074	−0.055 (0.161)	0.73
$LnHcy_{v1}$ × Time	0.0095 (0.0345)	0.78	+0.013 (0.034)	0.70
CDT:	N = 1445, K = 1.9		*N* = 1445, K = 1.9	
$LnHcy_{v1}$	−0.127 (0.102)	0.22	−0.059 (0.100)	0.56
$LnHcy_{v1}$ × Time	−0.045 (0.026)	0.087	−0.045 (0.026)	0.087
Log_e (TRAILS A):	N = 1428, K = 1.9		*N* = 1428, K = 1.9	
$LnHcy_{v1}$	+0.101 (0.031)	0.001[d]	+ 0.083 (0.031)	0.007
$LnHcy_{v1}$ × Time	−0.008 (0.007)	0.26[e]	−0.009 (0.009)	0.21[e]
Log_e(TRAILS B):	*N* = 1414, K = 1.8		N = 1414, K = 1.8	
$LnHcy_{v1}$	+0.116 (0.055)	0.034[e]	+0.059 (0.051)	0.25
$LnHcy_{v1}$ × Time	+0.009 (0.010)	0.36	+0.010 (0.010)	0.32
MEN: [c]				
MMSE, normalized:	N = 606, K = 1.8		*N* = 606, K = 1.8	
$LnHcy_{v1}$	−1.846 (2.035)	0.364	+1.894 (1.726)	0.273
$LnHcy_{v1}$ × Time	−0.029 (0.443)	0.947	−0.130 (0.434)	0.765
CVLT-List A:	*N* = 596, K = 1.7		N = 596, K = 1.7	
$LnHcy_{v1}$	+0.120 (0.813)	0.883	+0.934 (0.756)	0.217
$LnHcy_{v1}$ × Time	−0.137 (0.162)	0.400	−0.106 (0.165)	0.519
CVLT-DFR:	*N* = 578, K = 1.7		N = 578, K = 1.7	
$LnHcy_{v1}$	+0.061 (0.379)	0.872	+0.467 (0.359)	0.193
$LnHcy_{v1}$ × Time	+0.030 (0.082)	0.712	+0.027 (0.084)	0.746
BVRT:	*N* = 608, K = 1.9		N = 608, K = 1.9	
$LnHcy_{v1}$	+0.280 (0.591)	0.636	−0.363 (0.555)	0.513
$LnHcy_{v1}$ × Time	+0.297 (0.115)	0.010	+0.276 (0.115)	0.017
BTA:	*N* = 597, K = 1.7		N = 597, K = 1.7	
$LnHcy_{v1}$	−0.700 (0.270)	0.009	−0.446 (0.258)	0.084
$LnHcy_{v1}$ × Time	−0.000 (0.062)	0.995	+0.025 (0.063)	0.687
AF:	*N* = 613, K = 1.9		N = 613, K = 1.9	
$LnHcy_{v1}$	+0.102 (0.684)	0.882	+0.826 (0.654)	0.206
$LnHcy_{v1}$ × Time	−0.159 (0.122)	0.192	−0.134 (0.125)	0.284
DS-F:	N = 613, K = 1.9		N = 613, K = 1.9	
$LnHcy_{v1}$	−0.519 (0.274)	0.058	−0.046 (0.251)	0.853
$LnHcy_{v1}$ × Time	+0.002 (0.048)	0.968	+0.022 (0.049)	0.659
DS-B:	N = 613, K = 1.9		N = 613, K = 1.9	
$LnHcy_{v1}$	−0.504 (0.266)	0.059	−0.026 (0.240)	0.912
$LnHcy_{v1}$ × Time	+0.001 (0.050)	0.984	+0.003 (0.051)	0.948
CDT:	*N* = 610, K = 1.9		N = 610, K = 1.9	
$LnHcy_{v1}$	−0.042 (0.150)	0.781	+0.082 (0.148)	0.579
$LnHcy_{v1}$ × Time	−0.065 (0.039)	0.093	−0.067 (0.039)	0.089
Log_e (TRAILS A):	N = 598, K = 1.9		*N* = 598, K = 1.9	
$LnHcy_{v1}$	+0.082 (0.048)	0.089	+0.058 (0.048)	0.231
$LnHcy_{v1}$ × Time	+0.008 (0.011)	0.469	+0.002 (0.011)	0.839
Log_e(TRAILS B):	*N* = 590, K = 1.8		N = 590, K = 1.8	
$LnHcy_{v1}$	+0.263 (0.078)	0.001[d]	+0.147 (0.072)	0.040
$LnHcy_{v1}$ × Time	+0.017 (0.014)	0.220	+0.016 (0.014)	0.252

Table 2 (*continued*)

OVERALL:[c]	Plasma Homocysteine			
	Model 1[a]		Model 2[b]	
	β (SE)	*P* value	β (SE)	P value
WOMEN: [c]				
MMSE, normalized:	*N* = 824, K = 1.9		N = 824, K = 1.9	
$LnHcy_{v1}$	−1.863 (1.678)	0.267	−0.873 1.481437	0.556
$LnHcy_{v1}$ × Time	+0.216 (0.372)	0.561	+0.146 (0.371)	0.694
CVLT-List A:	N = 824, K = 1.7		N = 824, K = 1.7	
$LnHcy_{v1}$	+0.857 (0.796)	0.282	+1.257 (0.744)	0.091
$LnHcy_{v1}$ × Time	−0.074 (0.162)	0.649	−0.072 (0.163)	0.661
CVLT-DFR:	*N* = 813, K = 1.7		*N* = 813, K = 1.7	
$LnHcy_{v1}$	+0.444 (0.374)	0.235	+0.591 (0.359)	0.100
$LnHcy_{v1}$ × Time	−0.049 (0.078)	0.529	−0.041 (0.078)	0.594
BVRT:	*N* = 835, K = 1.9		N = 835, K = 1.9	
$LnHcy_{v1}$	+0.676 (0.554)	0.222	+0.461 (0.530)	0.384
$LnHcy_{v1}$ × Time	−0.005 (0.114)	0.967	−0.032 (0.115)	0.784
BTA:	*N* = 821, K = 1.8		N = 821, K = 1.8	
$LnHcy_{v1}$	+0.158 (0.277)	0.569	+0.190 (0.271)	0.483
$LnHcy_{v1}$ × Time	−0.025 (0.059)	0.677	−0.007 (0.060)	0.903
AF:	*N* = 833, K = 1.9		N = 833, K = 1.9	
$LnHcy_{v1}$	−1.143 (0.597)	0.055	−0.837 (0.572)	0.143
$LnHcy_{v1}$ × Time	+0.083 (0.113)	0.463	+0.048 (0.114)	0.672
DS-F:	*N* = 830, K = 1.9		N = 830, K = 1.9	
$LnHcy_{v1}$	−0.101 (0.245)	0.680	−0.083 (0.226)	0.713
$LnHcy_{v1}$ × Time	−0.049 (0.046)	0.286	−0.039 (0.047)	0.409
DS-B:	*N* = 831, K = 1.9		N = 831, K = 1.9	
$LnHcy_{v1}$	−0.183 (0.243)	0.453	−0.109 (0.222)	0.624
$LnHcy_{v1}$ × Time	+0.020 (0.048)	0.678	+0.043 (0.048)	0.375
CDT:	N = 835, K = 1.9		N = 835, K = 1.9	
$LnHcy_{v1}$	−0.209 (0.140)	0.135	−0.198 (0.136)	0.146
$LnHcy_{v1}$ × Time	−0.027 (0.036)	0.453	−0.016 (0.036)	0.661
Log_e (TRAILS A):	N = 830, K = 1.9		N = 830, K = 1.9	
$LnHcy_{v1}$	+0.110 (0.040)	0.006	+0.098 (0.040)	0.015
$LnHcy_{v1}$ × Time	−0.019 (0.009)	0.039	−0.018 (0.010)	0.056
Log_e(TRAILS B):	N = 824, K = 1.9		N = 824, K = 1.9	
$LnHcy_{v1}$	+0.007 (0.075)	0.927	−0.020 (0.073)	0.783
$LnHcy_{v1}$ × Time	−0.001 (0.015)	0.954	+0.003 (0.015)	0.839
WHITE: [c]				
MMSE, normalized:	*N* = 620, K = 1.9		N = 620, K = 1.9	
$LnHcy_{v1}$	−0.585 (2.074)	0.778	+2.193 (1.651)	0.184
$LnHcy_{v1}$ × Time	−0.372 (0.456)	0.414	−0.394 (0.449)	0.380
CVLT-List A:	*N* = 615, K = 1.7		N = 615, K = 1.7	
$LnHcy_{v1}$	−0.213 (0.989)	0.829	+0.497 (0.895)	0.579
$LnHcy_{v1}$ × Time	−0.128 (0.208)	0.537	−0.094 (0.207)	0.651
CVLT-DFR:	*N* = 599, K = 1.6		N = 599, K = 1.6	
$LnHcy_{v1}$	+0.284 (0.455)	0.532	+0.531 (0.425)	0.212
$LnHcy_{v1}$ × Time	−0.028 (0.100)	0.783	+0.004 (0.099)	0.971
BVRT:	*N* = 625, K = 1.9		N = 625, K = 1.9	
$LnHcy_{v1}$	+0.647 (0.573)	0.259	−0.028 (0.511)	0.956
$LnHcy_{v1}$ × Time	+0.060 (0.113)	0.598	+0.062 (0.115)	0.592
BTA:	*N* = 612, K = 1.7		N = 612, K = 1.7	
$LnHcy_{v1}$	−0.502 (0.297)	0.091	−0.299 (0.285)	0.293
$LnHcy_{v1}$ × Time	−0.019 (0.069)	0.788	−0.006 (0.070)	0.927
AF:	N = 625, K = 1.9		N = 625, K = 1.9	
$LnHcy_{v1}$	−1.666 (0.760)	0.028	−0.941 (0.703)	0.181
$LnHcy_{v1}$ × Time	+0.172 (0.150)	0.251	+0.168 (0.151)	0.268
DS-F:	*N* = 623, K = 1.9		N = 623, K = 1.9	
$LnHcy_{v1}$	−0.455 (0.308)	0.140	−0.083 (0.267)	0.756
$LnHcy_{v1}$ × Time	−0.013 (0.060)	0.826	−0.003 (0.060)	0.962
DS-B:	N = 625, K = 1.9		N = 625, K = 1.9	
$LnHcy_{v1}$	−0.436 (0.314)	0.165	−0.019 (0.273)	0.944
$LnHcy_{v1}$ × Time	−0.043 (0.059)	0.468	−0.040 (0.059)	0.504
CDT:	*N* = 626, K = 1.9		N = 626, K = 1.9	
$LnHcy_{v1}$	−0.199 (0.152)	0.191	−0.112 (0.150)	0.457
$LnHcy_{v1}$ × Time	−0.018 (0.042)	0.673	−0.018 (0.043)	0.678
Log_e (TRAILS A):	*N* = 619, K = 1.9		N = 619, K = 1.9	
$LnHcy_{v1}$	+0.089 (0.043)	0.039	+0.061 (0.042)	0.148
$LnHcy_{v1}$ × Time	−0.014 (0.010)	0.153	−0.014 (0.010)	0.138
Log_e(TRAILS B):	N = 615, K = 1.9		N = 615, K = 1.9	
$LnHcy_{v1}$	+0.100 (0.077)	0.194	+0.007 (0.069)	0.918
$LnHcy_{v1}$ × Time	−0.000 (0.013)	0.994	+0.002 (0.013)	0.885
AFRICAN AMERICAN: [c]				

(*continued on next page*)

Table 2 (*continued*)

OVERALL:[c]	Plasma Homocysteine			
	Model 1[a]		Model 2[b]	
	β (SE)	*P* value	β (SE)	P value
MMSE, normalized:	*N* = 810, K = 1.8		N = 810, K = 1.8	
$LnHcy_{v1}$	−2.383 (1.663)	0.152	−0.958 (1.503)	0.524
$LnHcy_{v1}$ × Time	+0.374 (0.365)	0.306	+0.285 (0.364)	0.433
CVLT-List A:	*N* = 805, K = 1.8		N = 805, K = 1.8	
$LnHcy_{v1}$	+0.932 (0.686)	0.174	+1.557(0.650)	0.017
$LnHcy_{v1}$ × Time	−0.078 (0.138)	0.573	−0.055 (0.140)	0.696
CVLT-DFR:	*N* = 792, K = 1.7		N = 792, K = 1.7	
$LnHcy_{v1}$	+0.232 (0.328)	0.479	+0.515 (0.320)	0.108
$LnHcy_{v1}$ × Time	+0.003 (0.068)	0.967	−0.007 (0.070)	0.924
BVRT:	*N* = 818, K = 1.9		N = 818, K = 1.9	
$LnHcy_{v1}$	+0.334 (0.556)	0.549	−0.056 (0.548)	0.918
$LnHcy_{v1}$ × Time	+0.192 (0.111)	0.083	+0.157 (0.111)	0.159
BTA:	*N* = 806, K = 1.8		N = 806, K = 1.8	
$LnHcy_{v1}$	−0.096 (0.258)	0.709	+0.053 (0.252)	0.832
$LnHcy_{v1}$ × Time	−0.012 (0.054)	0.824	−0.008 (0.054)	0.885
AF:	N = 821, K = 1.9		N = 821, K = 1.9	
$LnHcy_{v1}$	+0.153 (0.546)	0.779	+0.431 (0.536)	0.421
$LnHcy_{v1}$ × Time	−0.144 (0.098)	0.139	−0.146 (0.100)	0.142
DS-F:	*N* = 820, K = 1.9		N = 820, K = 1.9	
$LnHcy_{v1}$	−0.173 (0.224)	0.442	−0.015 (0.215)	0.944
$LnHcy_{v1}$ × Time	−0.026 (0.040)	0.512	−0.025 (0.040)	0.538
DS-B:	N = 819, K = 1.9		N = 819, K = 1.9	
$LnHcy_{v1}$	−0.250 (0.214)	0.244	−0.089 (0.198)	0.651
$LnHcy_{v1}$ × Time	+0.043 (0.042)	0.305	+0.051 (0.043)	0.227
CDT:	N = 819, K = 1.9		N = 819, K = 1.9	
$LnHcy_{v1}$	−0.093 (0.139)	0.501	−0.037 (0.137)	0.787
$LnHcy_{v1}$ × Time	−0.061 (0.034)	0.073	−0.056 (0.034)	0.101
Log_e (TRAILS A):	*N* = 809, K = 1.9		N = 809, K = 1.9	
$LnHcy_{v1}$	+0.094 (0.044)	0.032	+0.081 (0.044)	0.063
$LnHcy_{v1}$ × Time	−0.002 (0.010)	0.842	−0.007 (0.010)	0.502
Log_e(TRAILS B):	*N* = 799, K = 1.8		N = 799, K = 1.8	
$LnHcy_{v1}$	+0.116 (0.075)	0.125	+0.064 (0.072)	0.376
$LnHcy_{v1}$ × Time	+0.017 (0.015)	0.247	+0.016 (0.015)	0.299

Abbreviations: Hcy = Homocysteine; K = Mean number of visits per subject; Ln or Log_e = Loge transformed; N=Sample size; SE = Standard error; v_1 = visit 1.

[a] Model 1 is adjusted for age, sex, race, poverty status, inverse mills ratio as well as time on study between visits 1 and 2 (in years) and its interaction with blood homocysteine exposure $LnHcy_{v1}$ and covariates.

[b] Model 2 is adjusted for age, sex, race, poverty status, education, literacy, smoking, drug use, 2010 healthy eating index, body mass index, inverse mills ratio as well as time on study between visits 1 and 2 (in years) and its interaction with $LnHcy_{v1}$ and covariates.

[c] Cognitive tests include the Mini-Mental State Examination (MMSE), the California Verbal Learning Test (CVLT) Immediate (List A) and Delayed Free Recall (DFR), the Benton Visual Retention Test (BVRT, # of errors), the Brief Test of Attention (BTA), the Animal Fluency test (AF), the Digit Span Forward and Backwards tests (DS-F and DS-B), the Clock Drawing Test (CDT), the Trail making test Part A and B (TRAILS A and B, in seconds). K = mean observations/participant.

[d] $P < 0.05$ after familywise Bonferroni correction for main effect; $P < 0.10$ after familywise Bonferroni correction for 2-way interaction (Model 1).

[e] $P < 0.05$ for null hypothesis that $\gamma = 0$ for 2-way or 3-way interaction between sex, main Hcy exposure and TIME, in the unstratified mixed-effects linear regression model which included main effects of sex, Hcy exposure and TIME among others along with 2-way interaction terms between exposure, covariates and TIME.

[f] $P < 0.05$ for null hypothesis that $\gamma = 0$ for 2-way or 3-way interaction between race, main Hcy exposure and TIME, in the unstratified mixed-effects linear regression model which included main effects of race, Hcy exposure and TIME among others along with 2-way interaction terms between exposure, covariates and TIME.

3.3. Stratified analysis by sex and by race

For both LnHcy exposures ($LnHcy_{v1}$ and Hcy_{traj}), there was evidence of heterogeneity of their association with cognitive performance, both cross-sectionally and longitudinally across sex and race. In terms of sex differences, $LnHcy_{v1}$'s association with longitudinal performance on BVRT and Log_eTRAILSA and with cross-sectional performance on Log_eTRAILSB differed between men and women. The former 3-way interaction terms retained their statistical significance in *Model 2*. More specifically, among men, and unlike among women, $LnHcy_{v1}$ was associated with faster decline on the BVRT, a measure of visuo-spatial ability (β (SE): 0.297(0.115), $P = 0.010$, *Model 1*). Heterogeneity by race was also found with respect to $LnHcy_{v1}$ vs. AF score, cross-sectionally in *Model 1*. Specifically, among White adults $LnHcy_{v1}$ was associated with poorer baseline performance on AF (β (SE): −1.666 (0.760), $p = 0.028$), an association not detected among African American adults. This relationship was, however, attenuated in *Model 2*.

Similarly, with respect to Hcy_{traj}, a probable elevated LnHcy with age was linked to faster increase in the score on Log_eTRAILSB, reflecting a faster rate of decline, with significant heterogeneity across racial groups both in *Model 1* and *Model 2*. Specifically, unlike among White adults, among African American adults, an elevated and increasing LnHcy over time was associated with faster rate of decline on Log_eTRAILSB (β (SE): +0.012 (0.005), $p = 0.008$). Other notable stratum-specific findings were detected. Most notably, the "High increasing Hcy" trajectory was associated with better baseline verbal memory among women, both in terms of higher baseline scores on CVLT-List A (Model 1: β (SE): 0.837 (0.309), $p = 0.007$) and CVLT-DFR (Model 1: β (SE): 0.516 (0.146), $p < 0.001$), indicating that a better baseline verbal memory can predict an increasing trend in Hcy over time. This pattern was not observed among men.

3.4. Interactions with serum folate and B-12

Mixed-effects linear regression models were also conducted to test the interactive effects of the two Hcy exposures with serum folate and B-12 on longitudinal cognitive change. Our findings with respect to $LnHcy_{v1}$ suggested that simultaneous increase in $LnHcy_{v1}$ and serum folate was associated with faster decline on both BVRT and TRAILS A (Models 1 and 2, Supplementary Table 2). This pattern was also observed for clock command and Hcy_{traj} (Supplementary Table 3). In contrast, simultaneous increase in $LnHcy_{v1}$ and serum vitamin B-12 was associated with slower decline on BVRT (*Models 1* and *2*, Supplementary Table 2). No notable patterns of interaction were observed between Hcy_{traj} and serum vitamin B-12 with respect to change in cognition over time, especially after adjustment for key potential confounders in *Model 2*.

4. Discussion

The present study aimed to understand the association between Hcy levels and cognitive performance in a cohort of middle-aged African American and White adults. The study found that greater $LnHcy_{v1}$ was significantly associated with poorer baseline attention based on higher Log_e (TRAILS A, in seconds) [β (SE): 0.101 (0.031), $P = 0.001$]. Heterogeneity was also found by sex and by race. Most notably, among men only, $LnHcy_{v1}$ was associated with faster decline on the BVRT (# of errors), a measure of visuo-spatial memory (β (SE): 0.297(0.115), P = 0.010, reduced model); while among African American adults only, an elevated and increasing LnHcy over time was associated with faster rate of decline on Log_e (TRAILS B, in seconds) [β (SE): +0.012 (0.005), p = 0.008], a measure of executive function. Interactions between Hcy, folate and vitamin B-12 blood exposures were also detected.

A summary of the One Carbon Metabolism is provided in Supplementary Material 5. Hcy has been found to be a likely risk factor for dementia spectrum disorders (Ansari et al., 2014). More specifically, increased Hcy levels have been associated with a number of mental symptoms, including dementia, and cognitive impairment (Kim and Lee, 2014).In a comprehensive review and meta-analysis of selected modifiable risk factors, incident AD was associated mainly with 3 of those risk factors, namely low education, elevated Hcy and reduced physical

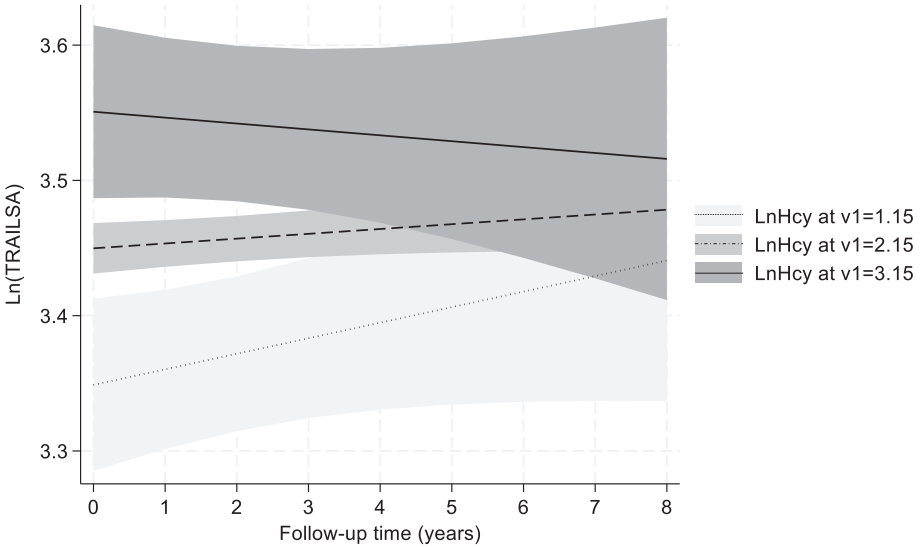


Fig. 3. Predictive margins of Ln(TRAILS A) vs. follow-up time across selected levels of $LnHcy_{v1}$ – HANDLS (2004–2013).
Notes: HANDLS = Healthy Aging in Neighborhoods of Diversity across the Lifespan Study; Hcy = Homocysteine; Ln = natural logarithm, Log_e; TRAILS A = Trailmaking Test, part A; v1 = Visit 1. Predictive margins are based on mixed-effects linear regression models with Ln(TRAILS A) as the outcome, and $LnHcy_{v1}$ as the main exposure interacted with time at follow-up. Model is also adjusted for age, sex, race, poverty status and the inverse mills ratio which were also interacted with time (Model 1, Table 2). Levels of $LnHcy_{v1}$ are based on mean centered values, subtracting and adding 1. These correspond to 3.2, 8.6 and 23.3 mg/mL, respectively.

activity (Beydoun et al., 2014). To determine whether a percentage of dementia worldwide might be avoided, extensive randomized trials with vitamins that decrease Hcy are required, particularly folate, vitamin B-12 (i.e. cobalamin) and vitamin B-6 (Smith, 2008).

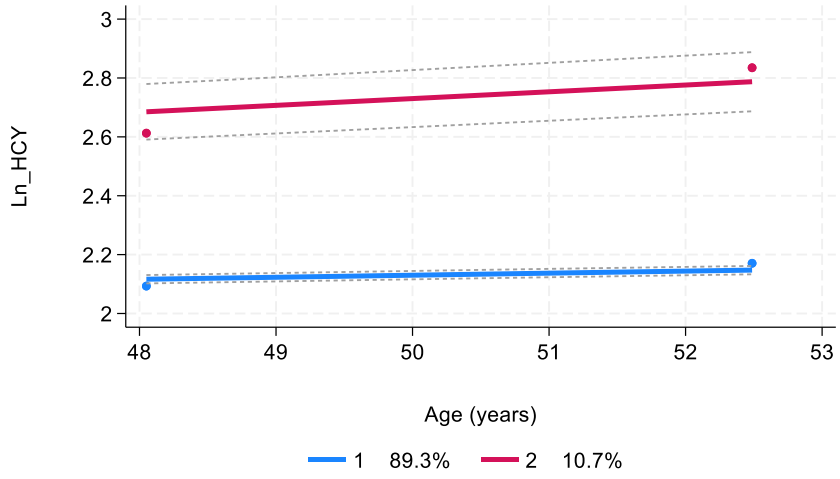
High blood Hcy levels are a risk factor for diseases involving different B vitamins, including AD, vascular dementia, frontotemporal dementia, and Lewy body dementia (Song et al., 2022). Given that vitamin B12 and folate are cofactors necessary for Hcy methylation, vitamin B-12 or folate deficiency can increase Hcy levels. How cognitive function responds with the interactions between Hcy, Vitamin B-12, and folate remains inconclusive. High blood Hcy levels constitute a risk factor for diseases involving different B vitamins, including AD, vascular dementia, frontotemporal dementia, and Lewy body dementia (Song et al., 2022). A study found that cognitive test scores were only positively associated with Hcy levels in mildly cognitive impaired individuals (Song et al., 2022). Yet scores were negatively associated with vitamin B-12 levels in both mildly cognitively impaired and vascular demented persons (Song et al., 2022). Both Hcy and vitamin B-12 levels were associated with Fazekas and temporal lobe atrophy (MTA) in AD and general cognition in vascular dementia (VaD) (Song et al., 2022). A case-control study in older Chinese adults found that serum folate and vitamin B-12 levels were significantly lower in patients with MCI and AD, but plasma Hcy levels were higher (Ma et al., 2017). No association existed between low vitamin B12 levels and AD or MCI (Ma et al., 2017).

Similarly, research indicates a possible role of vitamin D in cognitive function, with studies examining the relationships between Hcy, vitamins D, B12, and folate with cognition. A study found that vitamin D deficiency, low folate levels, and high homocysteine levels are more pronounced in subcortical vascular dementia (sVAD) cases than in Alzheimer's disease (AD) (Moretti et al., 2017). Vitamin B-12 and Hcy in plasma may also interact antagonistically with regard to age-related cognitive decline, while folate, vitamins B-6, and B-12 may prevent cognitive decline and postpone the onset of dementia(Duthie et al., 2002; Feng et al., 2006; Haan et al., 2007; Kado et al., 2005; Li et al., 2008; Mooijaart et al., 2005; Ramos et al., 2005; Ravaglia et al., 2005; Tucker et al., 2005; Vidal et al., 2008). In fact, a prospective cohort study from the Singapore Longitudinal Aging Study (SLAS-2) found that 5.7 % of cognitively normal participants, aged ≥55 years of age, developed neurocognitive disorders (NCD) at 4.5 y follow-up (Przybycien-Gaweda et al., 2022). Low serum B12 in the presence of high serum folate, low serum vitamin B12, and high Hcy were independently significantly associated with incident NCD(Przybycien-Gaweda et al., 2022). Furthermore, folate and cobalamin have been linked to increased brain volume, particularly in the hippocampus and amygdala areas, as well as decreased white matter lesion severity(de Lau et al., 2009; Pieters et al., 2009). Vitamin B-6 and cobalamin intakes have also been demonstrated to protect against gray matter (GM) atrophy, with a specific link between cobalamin status and bi-lateral superior parietal sulcus(Erickson et al., 2008). Recent research using a sub-sample of HANDLS (HANDLS SCAN) found a link between higher levels of cobalamin and larger volumes of the inferior frontal gyrus (Beydoun et al., 2020d), which is renowned for its role in speech and language processing (Greenlee et al., 2007).

More importantly, a large recent randomized controlled trial (VITACOG) conducted among MCI patients revealed that high-dose B vitamin supplementation benefited GM regions vulnerable to AD by slowing atrophy rates over two years, though this only applied to hyperhomocysteinemic individuals (Douaud et al., 2013). This trial indicated that B vitamin supplementation can stabilize executive functions and reduce decline in global cognition, episodic and semantic memory(de Jager et al., 2012). A meta-analysis of 23 randomized-controlled trials compared the cognitive function of adults over 50 years of age with or without impaired cognition who took B-vitamins including Vitamin B_6 and B_{12} or folic acid supplementation with placebo (Chang et al., 2023). The data indicated B-vitamins and/or folate supplementation versus placebo significantly reduced homocysteine levels (Chang et al., 2023). However, there were no significant differences in scores of cognitive function - Mini Mental State Examination or Clinical Dementia Rating, between the supplementation and placebo groups suggesting supplementation failed to provide any benefits over placebo in preventing or slowing the decline in cognitive function (Chang et al., 2023).

This study on the relationship between cognitive performance and health has several strengths including the prospective cohort design that allowed for stratification by sex and by race groups, the use of repeat measures on both exposures and outcomes, and the additional analyses on interactions between Hcy, folate and vitamin B-12 blood exposures. The large number of cognitive test scores allowed us to explore a large

(A)



(B)

	β	SE	P
Group 1			
Intercept	**+1.78122**	**0.02982**	**<0.001**
Linear Age	**+0.00698**	**0.00057**	**<0.001**
Group 2			
Intercept	**+1.57905**	**0.20475**	**<0.001**
Linear Age	**+0.02302**	**0.00413**	**<0.001**

Fig. 2. Group-based trajectories for plasma homocysteine – HANDLS (2004–2013).
Notes: HANDLS = Healthy Aging in Neighborhoods of Diversity across the Lifespan Study;
A = Graphical display of two groups identified using group-based trajectory modeling, whereby HCY represents Log_e transformed plasma homocysteine and Age (years) represents the time variable. B = Table display of intercept and linear terms for the two trajectories in HCY identified using group-based trajectories, whereby Group 1 plasma homocysteine is lower and increases significantly over time and Group 2 plasma homocysteine is higher and increases significantly with age. *N* = 1532 used in the GBTM model.

number of inter-twined cognitive domains rather than focusing only on cognitive status or a few domains of interest Nevertheless, the study may have been biased due to the use of a sub-sample from the initial HANDLS participants, and measurement error might have remained, potentially leading to skewed assessments. A significant shift in cognitive performance is less likely to be detected over the two-visit follow-up period, so future research should investigate correlations over extended periods. Residual confounding is probable due to the observational, prospective cohort study, making it impossible to demonstrate causality. The study included almost equal numbers of White and African American individuals, men and women, but an analysis of interaction effects between sex and race may have been underpowered. Furthermore, evidence from multi-group analyses suggests that the data reduction through factor analysis did not achieve group invariance across sex or race. Thus, we opted to use all 11 cognitive test scores as opposed to conducting dimensionality reduction Finally, the sampling approach used in the study may not generalize the findings to other populations.

In summary, sex- and race-specific adverse association between elevated Hcy and cognitive performance over time were detected among middle-aged urban adults, in domains of attention, visuo-spatial memory and executive functioning. The relationship may be bidirectional, potentially affecting risk factor management and Hcy course. However, longitudinal associations were only found for specific domains in men and African American adults.

Funding

This research was supported entirely by the Intramural Research Program of the NIH, National Institute on Aging (Z01-AG000513).

CRediT authorship contribution statement

May A. Beydoun: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Hind A. Beydoun:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis, Conceptualization. **Michael F. Georgescu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Data curation, Conceptualization. **Christian A. Maino Vieytes:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Conceptualization. **Marie T. Fanelli-Kuczmarski:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration,

Table 3
Relationship of blood homocysteine trajectory based on group-based trajectory models of LnHcy with age (Hcy $_{traj}$) with 11 cognitive test scores (baseline and between-visit change), overall, and by stratifying variables: HANDLS 2004–2013.

	Plasma Homocysteine Trajectory			
	Model 1[a]		Model 2[b]	
	β (SE)	P	β (SE)	P
OVERALL:[c]				
MMSE, normalized:	*N* = 1398, K = 1.9		N = 1398, K = 1.9	
Hcy $_{traj}$	−0.357 (0.417)	0.39	+0.063 (0.358)	0.86
Hcy $_{traj}$ × Time	+0.017 (0.087)	0.84	+0.008 (0.085)	0.92
CVLT-List A:	N = 1391, K = 1.8		N = 1391, K = 1.8	
Hcy $_{traj}$	+0.371 (0.184)	0.044[e]	+0.478 (0.172)	0.005
Hcy $_{traj}$ × Time	−0.054 (0.036)	0.13	−0.053 (0.036)	0.14
CVLT-DFR:	*N* = 1365, K = 1.7		N = 1365, K = 1.7	
Hcy $_{traj}$	+0.271 (0.087)	0.002[d,e]	+0.314 (0.083)	<0.001
Hcy $_{traj}$ × Time	−0.035 (0.018)	0.049	−0.034 (0.018)	0.054
BVRT:	N = 1412, K = 1.9		N = 1412, K = 1.9	
Hcy $_{traj}$	+0.034 (0.130)	0.79	−0.051 (0.123)	0.68
Hcy $_{traj}$ × Time	+0.058 (0.025)	0.022	+0.057 (0.024)	0.024
BTA:	*N* = 1392, K = 1.8		N = 1392, K = 1.8	
Hcy $_{traj}$	−0.048 (0.061)	0.43	−0.015 (0.058)	0.80
Hcy $_{traj}$ × Time	−0.019 (0.013)	0.14	−0.019 (0.013)	0.14
AF:	N = 1414, K = 1.9		N = 1414, K = 1.9	
Hcy $_{traj}$	−0.156 (0.145)	0.28	−0.046 (0.138)	0.74
Hcy $_{traj}$ × Time	−0.034 (0.026)	0.18	−0.034 (0.026)	0.18
DS-F:	N = 1412, K = 1.9		N = 1412, K = 1.9	
Hcy $_{traj}$	−0.078 (0.058)	0.18	−0.021 (0.053)	0.69
Hcy $_{traj}$ × Time	+0.014 (0.010)	0.16	+0.014 (0.010)	0.17
DS-B:	*N* = 1413, K = 1.9		N = 1413, K = 1.9	
Hcy $_{traj}$	−0.111 (0.057)	0.054	−0.047 (0.051)	0.35
Hcy $_{traj}$ × Time	+0.004 (0.011)	0.72	+0.003 (0.011)	0.76
CDT:	N = 1414, K = 1.9		*N* = 1414, K = 1.9	
Hcy $_{traj}$	−0.055 (0.033)	0.095	−0.036 (0.032)	0.25
Hcy $_{traj}$ × Time	−0.009 (0.008)	0.30	−0.010 (0.008)	0.23
Log_e (TRAILS A):	*N* = 1397, K = 1.9		N = 1397, K = 1.9	
Hcy $_{traj}$	+0.032 (0.0099)	0.001[d]	+0.030 (0.010)	0.002
Hcy $_{traj}$ × Time	−0.001 (0.002)	0.69[e]	−0.001 (0.002)	0.57
Log_e (TRAILS B):	*N* = 1383, K = 1.9		N = 1383, K = 1.9	
Hcy $_{traj}$	. + 0.029 (0.017)	0.095[e]	+0.019 (0.016)	0.24
Hcy $_{traj}$ × Time	+0.007 (0.003)	0.040[f]	+0.006 (0.003)	0.046[f]
MEN:[c]				
MMSE, normalized:	*N* = 586, K = 1.9		*N* = 586, K = 1.9	
Hcy $_{traj}$	−0.639 (0.562)	0.255	+0.337 (0.481)	0.483
Hcy $_{traj}$ × Time	+0.033 (0.117)	0.778	−0.029 (0.116)	0.803
CVLT-List A:	N = 578, K = 1.7		N = 578, K = 1.7	
Hcy $_{traj}$	+0.029 (0.226)	0.898	+0.261 (0.211)	0.217
Hcy $_{traj}$ × Time	−0.031 (0.044)	0.484	−0.029 (0.045)	0.524
CVLT-DFR:	*N* = 562, K = 1.7		N = 562, K = 1.7	
Hcy $_{traj}$	+0.099 (0.105)	0.347	+0.201 (0.101)	0.046
Hcy $_{traj}$ × Time	−0.026 (0.022)	0.245	−0.029 (0.023)	0.213
BVRT:	*N* = 589, K = 1.9		N = 589, K = 1.9	
Hcy $_{traj}$	+0.160 (0.164)	0.329	−0.016 (0.154)	0.918
Hcy $_{traj}$ × Time	+0.077 (0.031)	0.014	+0.074 (0.031)	0.018
BTA:	*N* = 580, K = 1.8		N = 580, K = 1.8	
Hcy $_{traj}$	−0.131 (0.075)	0.080	−0.069 (0.072)	0.338
Hcy $_{traj}$ × Time	−0.021 (0.017)	0.195	−0.014 (0.017)	0.390
AF:	*N* = 593, K = 1.9		N = 593, K = 1.9	
Hcy $_{traj}$	−0.165 (0.191)	0.388	+0.017 (0.184)	0.928
Hcy $_{traj}$ × Time	−0.044 (0.033)	0.185	−0.041 (0.034)	0.237
DS-F:	N = 593, K = 1.9		N = 593, K = 1.9	
Hcy $_{traj}$	−0.078 (0.075)	0.300	+0.036 (0.070)	0.602
Hcy $_{traj}$ × Time	−0.015 (0.013)	0.227	−0.010 (0.013)	0.465
DS-B:	*N* = 594, K = 1.9		N = 594, K = 1.9	
Hcy $_{traj}$	−0.160 (0.073)	0.029	−0.048 (0.066)	0.472
Hcy $_{traj}$ × Time	+0.000 (0.013)	1.000	+0.003 (0.013)	0.812
CDT:	*N* = 591, K = 1.9		N = 591, K = 1.9	
Hcy $_{traj}$	−0.062 (0.041)	0.133	−0.038 (0.041)	0.359
Hcy $_{traj}$ × Time	−0.013 (0.010)	0.223	−0.011 (0.011)	0.278
Log_e (TRAILS A):	*N* = 579, K = 1.9		N = 579, K = 1.9	
Hcy $_{traj}$	+0.031 (0.013)	0.019	+0.025 (0.013)	0.061
Hcy $_{traj}$ × Time	+0.003 (0.003)	0.289	+0.002 (0.003)	0.573
Log_e (TRAILS B):	*N* = 571, K = 1.9		N = 571, K = 1.9	
Hcy $_{traj}$	+0.067 (0.021)	0.002	+0.042 (0.020)	0.031
Hcy $_{traj}$ × Time	+0.007 (0.004)	0.080	+0.007 (0.004)	0.086
WOMEN:[c]				
MMSE, normalized:	*N* = 812, K = 1.9		N = 812, K = 1.9	
Hcy $_{traj}$	−0.087 (0.652)	0.893	−0.296 (0.575)	0.607
Hcy $_{traj}$ × Time	+0.017 (0.137)	0.899	+0.061 (0.137)	0.659
CVLT-List A:	N = 813, K = 1.8		N = 813, K = 1.8	
Hcy $_{traj}$	+0.837 (0.309)	0.007	+0.827 (0.287)	0.004[d]
Hcy $_{traj}$ × Time	−0.091 (0.061)	0.135	−0.088 (0.061)	0.147
CVLT-DFR:	*N* = 803, K = 1.7		N = 803, K = 1.7	
Hcy $_{traj}$	+0.516 (0.146)	<0.001[d]	+0.511 (0.139)	<0.001[d]
Hcy $_{traj}$ × Time	−0.052 (0.029)	0.078	−0.051 (0.029)	0.083
BVRT:	*N* = 823, K = 1.9		N = 823, K = 1.9	
Hcy $_{traj}$	−0.088 (0.214)	0.682	−0.136 (0.203)	0.504
Hcy $_{traj}$ × Time	+0.023 (0.043)	0.589	+0.023 (0.043)	0.596
BTA:	N = 812, K = 1.8		N = 812, K = 1.8	
Hcy $_{traj}$	+0.062 (0.102)	0.546	+0.088 (0.099)	0.374
Hcy $_{traj}$ × Time	−0.011 (0.021)	0.602	−0.016 (0.022)	0.465
AF:	N = 821, K = 1.9		N = 821, K = 1.9	

(*continued on next page*)

Table 3 (*continued*)

	Plasma Homocysteine Trajectory			
	Model 1[a]		Model 2[b]	
	β (SE)	P	β (SE)	P
Hcy $_{traj}$	−0.223 (0.231)	0.336	−0.201 (0.221)	0.364
Hcy $_{traj}$ × Time	−0.016 (0.042)	0.708	−0.036 (0.042)	0.394
DS-F:	N = 819, K = 1.9		N = 819, K = 1.9	
Hcy $_{traj}$	−0.099 (0.094)	0.290	−0.111 (0.086)	0.198
Hcy $_{traj}$ × Time	−0.007 (0.017)	0.675	−0.007 (0.017)	0.667
DS-B:	N = 819 K = 1.9		*N* = 819, K = 1.9	
Hcy $_{traj}$	−0.041 (0.094)	0.660	−0.035 (0.086)	0.684
Hcy $_{traj}$ × Time	+0.011 (0.018)	0.517	+0.010 (0.018)	0.566
CDT:	N = 823, K = 1.9		N = 823, K = 1.9	
Hcy $_{traj}$	−0.060 (0.054)	0.270	−0.071 (0.053)	0.176
Hcy $_{traj}$ × Time	−0.002 (0.013)	0.910	+0.003 (0.013)	0.818
Log_e (TRAILS A):	N = 818, K = 1.9		N = 818, K = 1.9	
Hcy $_{traj}$	+0.034 (0.015)	0.028	+0.032 (0.015)	0.036
Hcy $_{traj}$ × Time	−0.006 (0.003)	0.063	−0.007 (0.003)	0.034
Log_e (TRAILS B):	N = 812, K = 1.9		N = 812, K = 1.9	
Hcy $_{traj}$	−0.024 (0.029)	0.401	−0.039 (0.027)	0.151
Hcy $_{traj}$ × Time	+0.006 (0.005)	0.285	+0.008 (0.006)	0.170
WHITE:[c]				
MMSE, normalized:	*N* = 605, K = 1.9		N = 605, K = 1.9	
Hcy $_{traj}$	−0.353 (0.689)	0.608	−0.174 (0.570)	0.760
Hcy $_{traj}$ × Time	+0.026 (0.147)	0.858	+0.057 (0.145)	0.694
CVLT-List A:	*N* = 602, K = 1.7		N = 602, K = 1.7	
Hcy $_{traj}$	+0.510 (0.321)	0.113	+0.522 (0.289)	0.070
Hcy $_{traj}$ × Time	−0.077 (0.064)	0.229	−0.059 (0.064)	0.362
CVLT-DFR:	N = 586, K = 1.7		N = 586, K = 1.7	
Hcy $_{traj}$	+0.408 (0.148)	0.006	+0.404 (0.138)	0.003[d]
Hcy $_{traj}$ × Time	−0.038 (0.031)	0.229	−0.029 (0.031)	0.362
BVRT:	N = 610, K = 1.9		N = 610, K = 1.9	
Hcy $_{traj}$	+0.082 (0.190)	0.667	−0.075 (0.167)	0.654
Hcy $_{traj}$ × Time	+0.048 (0.035)	0.175	+0.063 (0.036)	0.079
BTA:	*N* = 601, K = 1.8		N = 601, K = 1.8	
Hcy $_{traj}$	−0.133 (0.095)	0.160	−0.105 (0.090)	0.246
Hcy $_{traj}$ × Time	−0.015 (0.021)	0.475	−0.015 (0.021)	0.486
AF:	N = 610, K = 1.9		N = 610, K = 1.9	
Hcy $_{traj}$	−0.276 (0.255)	0.280	−0.187 (0.237)	0.430
Hcy $_{traj}$ × Time	−0.009 (0.049)	0.852	−0.015 (0.049)	0.753
DS-F:	*N* = 609, K = 1.9		N = 609, K = 1.9	
Hcy $_{traj}$	−0.153 (0.101)	0.131	−0.142 (0.088)	0.104
Hcy $_{traj}$ × Time	−0.010 (0.019)	0.591	−0.008 (0.019)	0.691
DS-B:	N = 610, K = 1.9		N = 610, K = 1.9	
Hcy $_{traj}$	−0.126 (0.104)	0.225	−0.076 (0.091)	0.404
Hcy $_{traj}$ × Time	−0.007 (0.019)	0.719	−0.011 (0.019)	0.542
CDT:	*N* = 611, K = 1.9		N = 611, K = 1.9	

Table 3 (*continued*)

	Plasma Homocysteine Trajectory			
	Model 1[a]		Model 2[b]	
	β (SE)	P	β (SE)	P
Hcy $_{traj}$	−0.081 (0.050)	0.109	−0.053 (0.049)	0.272
Hcy $_{traj}$ × Time	−0.000 (0.013)	0.975	+0.000 (0.014)	0.985
Log_e (TRAILS A):	*N* = 604, K = 1.9		N = 604, K = 1.9	
Hcy $_{traj}$	+0.042 (0.014)	0.003[d]	+0.039 (0.014)	0.005
Hcy $_{traj}$ × Time	−0.003 (0.003)	0.369	−0.003 (0.003)	0.307
Log_e (TRAILS B):	*N* = 600, K = 1.9		N = 600, K = 1.9	
Hcy $_{traj}$	+0.040 (0.025)	0.117	+0.026 (0.023)	0.255
Hcy $_{traj}$ × Time	−0.002 (0.004)	0.659	−0.004 (0.004)	0.361
AFRICAN AMERICAN:[c]				
MMSE, normalized:	*N* = 793, K = 1.9		N = 793, K = 1.9	
Hcy $_{traj}$	−0.434 (0.523)	0.407	+0.033 (0.448)	0.942
Hcy $_{traj}$ × Time	+0.038 (0.110)	0.731	+0.025 (0.110)	0.820
CVLT-List A:	*N* = 789, K = 1.8		N = 789, K = 1.8	
Hcy $_{traj}$	+0.246 (0.221)	0.265	+0.402 (0.209)	0.054
Hcy $_{traj}$ × Time	−0.037 (0.043)	0.393	−0.026 (0.044)	0.552
CVLT-DFR:	*N* = 779, K = 1.7		N = 779, K = 1.7	
Hcy $_{traj}$	+0.163 (0.107)	0.127	+0.245 (0.103)	0.018
Hcy $_{traj}$ × Time	−0.031 (0.022)	0.157	−0.033 (0.022)	0.132
BVRT:	*N* = 802, K = 1.9		N = 802, K = 1.9	
Hcy $_{traj}$	+0.029 (0.175)	0.867	−0.106 (0.171)	0.534
Hcy $_{traj}$ × Time	+0.067 (0.034)	0.052	+0.058 (0.034)	0.088
BTA:	*N* = 791, K = 1.8		N = 791, K = 1.8	
Hcy $_{traj}$	−0.007 (0.080)	0.931	+0.041 (0.078)	0.598
Hcy $_{traj}$ × Time	−0.022 (0.017)	0.184	−0.018 (0.017)	0.279
AF:	*N* = 804, K = 1.9		N = 804, K = 1.9	
Hcy $_{traj}$	−0.103 (0.172)	0.547	−0.020 (0.169)	0.907
Hcy $_{traj}$ × Time	−0.049 (0.030)	0.104	−0.049 (0.031)	0.110
DS-F:	N = 803, K = 1.9		N = 803, K = 1.9	
Hcy $_{traj}$	−0.032 (0.070)	0.645	+0.036 (0.067)	0.590
Hcy $_{traj}$ × Time	−0.016 (0.012)	0.163	−0.016 (0.012)	0.181
DS-B:	N = 803, K = 1.9		N = 803, K = 1.9	
Hcy $_{traj}$	−0.107 (0.067)	0.111	−0.033 (0.061)	0.583
Hcy $_{traj}$ × Time	+0.012 (0.013)	0.360	+0.012 (0.013)	0.343
CDT:	N = 803, K = 1.9		N = 803, K = 1.9	
Hcy $_{traj}$	−0.045 (0.044)	0.301	−0.030 (0.043)	0.478
Hcy $_{traj}$ × Time	−0.013 (0.010)	0.202	−0.010 (0.010)	0.348
Log_e (TRAILS A):	N = 793, K = 1.9		N = 793, K = 1.9	
Hcy $_{traj}$	+0.023 (0.014)	0.098	+0.020 (0.014)	0.136
Hcy $_{traj}$ × Time	+0.001 (0.003)	0.718	−0.001 (0.003)	0.833
Log_e (TRAILS B):	*N* = 783, K = 1.8		N = 783, K = 1.8	
Hcy $_{traj}$	+0.022 (0.024)	0.353	+0.004 (0.022)	0.858
Hcy $_{traj}$ × Time	+0.012 (0.005)	0.008[d]	+0.012 (0.005)	0.008[d]

Abbreviations: Hcy = Homocysteine; Hcy $_{traj}$ = z-transformed probability of belonging to a group with elevated and/or increasing LnHcy over time according

to group-based trajectory modeling; K = Mean number of visits per subject; Ln or Log_e = Log_e transformed; N=Sample size; SE = Standard error.

[a] Model 1 is adjusted for age, sex, race, poverty status, inverse mills ratio as well as time on study in years between visits 1 and 2 and its interaction with Hcy_{traj} and covariates.

[b] Model 2 is adjusted for age, sex, race, poverty status, education, literacy, smoking, drug use, 2010 healthy eating index, body mass index, inverse mills ratio as well as time on study in years between visits 1 and 2 and its interaction with Hcy_{traj} and covariates.

[c] Cognitive tests include the Mini-Mental State Examination (MMSE), the California Verbal Learning Test (CVLT) Immediate (List A) and Delayed Free Recall (DFR), the Benton Visual Retention Test (BVRT, # of errors), the Brief Test of Attention (BTA), the Animal Fluency test (AF), the Digit Span Forward and Backwards tests (DS-F and DS-B), the Clock Drawing Test (CDT), the Trail making test Part A and B (TRAILS A and B, in seconds). K = mean observations/participant.

[d] $P < 0.05$ after familywise Bonferroni correction for main effect; $P < 0.10$ after familywise Bonferroni correction for 2-way interaction (Model 1).

[e] $P < 0.05$ for null hypothesis that $\gamma = 0$ for 2-way or 3-way interaction between sex, main Hcy exposure and TIME, in the unstratified mixed-effects linear regression model which included main effects of sex, Hcy exposure and TIME among others along with 2-way interaction terms between exposure, covariates and TIME.

[f] $P < 0.05$ for null hypothesis that $\gamma = 0$ for 2-way or 3-way interaction between race, main Hcy exposure and TIME, in the unstratified mixed-effects linear regression model which included main effects of race, Hcy exposure and TIME among others along with 2-way interaction terms between exposure, covariates and TIME.

Methodology, Investigation, Data curation, Conceptualization. **Nicole Noren Hooten:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Funding acquisition, Data curation, Conceptualization. **Michele K. Evans:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Alan B. Zonderman:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

All authors declare no conflict of interest.

(a) Financial disclosure: This work was supported in full by the Intramural research Program of the NIH, National institute on Aging.
(b) Non-financial disclosure: None declared.

Acknowledgements

The authors would like to thank staff, investigators and participants in HANDLS and NIA internal reviewers. Dr. Hind A. Beydoun worked on this manuscript outside her tour of duty at the U.S. Department of Veterans Affairs.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jad.2024.08.010.

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